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Biocide Activity against Urinary Catheter Pathogens

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Antimicrobial effects of essential oils against bacteria associated with urinary catheter infection was assessed. Tests were performed on 14 different bacterial species cultured either planktonically or as biofilms. Biofilms were found to be up to 8-fold more tolerant of the test agents. Higher antimicrobial tolerance was also evident in tests conducted in artificial urine. Eugenol exhibited higher antimicrobial effects against both planktonic cells and biofilms than did terpinen, tea tree oil, and cineole.

Foley catheters are frequently used to drain urine from the bladders of patients with urinary incontinence or neurological dysfunction. While providing a valuable function, urinary catheters also provide access for microorganisms to infect the bladder and also undermine the basic host defenses of the urinary tract. Importantly, catheter-associated urinary tract infections (CAUTIs) are the most frequently encountered hospital-acquired infections (1). *Proteus mirabilis* is a urease-positive bacterium that raises the urine pH during infection. This allows struvite and apatite crystal encrustation of the catheter, which obstructs urine flow, potentially promoting serious clinical complications. All currently available catheters are vulnerable to this encrustation, and there is no single effective preventative strategy (2–4).

Because of the increasing prevalence of antibiotic resistance, an interest in the therapeutic use of alternative medicines in combating infection has arisen (5–9). Naturally occurring biocides are effective in inactivating a wide variety of microorganisms, as they often target multiple bacterial sites and therefore are less prone to the development of resistance than are antibiotics (10, 11). One possible approach to the prevention of catheter encrustation is to incorporate these biocides into catheter washout solutions or into the catheter material itself or to use the catheter retention balloon as a reservoir for the delivery of the antimicrobial agent into the catheterized bladder (12–16). Little is known about the susceptibility of *Proteus* to natural antimicrobial agents; hence, the aim of this study was to examine antimicrobial activities of several essential oils, namely, tea tree oil (TTO), terpinen, cineole, and eugenol, against *P. mirabilis* and other urease-producing bacteria involved in CAUTIs. The activity of these agents against both planktonic cells and biofilms was tested, as the latter frequently exhibit enhanced resistance to traditional antimicrobials.

The isolates used in this study are presented in Table 1. The antimicrobial agents tested were cineole, TTO, terpinen, and eugenol. Overnight cultures of test isolates were prepared in Mueller-Hinton broth (MHB) (17) or artificial urine (AU) adjusted to pH 6.1 and containing 1% Tryptone soya broth (18). Cultures were adjusted to a 0.5 McFarland standard (approximately 10^8 cells ml^{-1}) and diluted 100-fold in MHB or AU. Serial dilutions of the antimicrobial agents were prepared in MHB or AU supplemented with 0.002% (vol/vol) Tween 80 (Sigma-Aldrich, UK). A 100-μl volume of each dilution of antimicrobial agent was added to an equal volume of a microbial suspension, giving antimicrobial concentrations ranging from 0.008 to 8% (vol/vol). Controls included bacterial suspensions containing no antimicrobial agent and uninoculated culture medium. The bacteria and antimicrobial agent were coincubated aerobically in 96-well microtiter plates for 24 h at 37°C. Microbial growth was determined by spectrophotometric analysis (620 nm). Absorbance readings were standardized against "microbial-free" antimicrobial agent controls. The MIC was defined as the lowest concentration of antimicrobial agent which resulted in a ≥80% reduction in absorbance compared to that of the antimicrobial-free controls (19).

A biofilm susceptibility test was also performed with the isolates described in Table 1. Isolates were incubated as described above but without agitation to allow biofilm formation. Culture medium was removed, and the biofilms were washed with 100 μl phosphate-buffered saline (PBS) to remove planktonic cells. Fresh culture medium (100 μl) containing an antimicrobial agent at concentrations ranging from 0.008 to 8% (vol/vol) was added to each well. Controls prepared as already described were also included. Biofilms were incubated in the presence of an antimicrobial agent for 24 h without agitation under the conditions described above before the supernatant was removed and the biofilm was washed once with PBS. Fresh culture medium (100 μl) that did not contain an antimicrobial agent was added to the biofilms, which were disrupted by repeated pipetting. The turbidity (620 nm) of the resuspended biofilm was measured and again after incubation at 37°C for an additional 6 and 24 h. The relative growth of microorganisms was determined by measuring the change in absorbance, and antibiofilm activity was recorded as the lowest concentration of an agent that demonstrated a ≥80% reduction in absorbance compared to that of the control. All experiments were performed in triplicate on three separate occasions in MHB and AU.

A summary of the results is presented in Table 1. Cineole and TTO had the lowest antimicrobial activity against bacteria grown in MHB and were therefore not tested in AU. The highest antimicrobial activity by the essential oils against planktonic growth in MHB was that of eugenol and terpinen. However, when terpinen was tested in AU, its activity was noticeably lower. The biofilms also showed a higher tolerance (up to 8-fold) to most of the essen-

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tial oils than did their planktonic equivalents. This increased biofilm resistance was least evident with eugenol in MHB but was apparent with eugenol (up to 32-fold) against biofilms in AU.

Urinary catheters provide a convenient means to drain urine from the bladders of patients suffering from urinary incontinence or neurological dysfunction. However, they are also associated with complications, as they provide access for bacteria from a heavily contaminated external skin site to the bladder and kidneys (20). Catheters also undermine the normal filling and emptying of the bladder, which flushes out microorganisms that might be contaminating the urethra. Furthermore, a reservoir of residual urine remains in the bladder of catheterized patients, allowing continued proliferation of contaminating organisms (21). CAUTIs are usually asymptomatic, and because of the danger of promoting antibiotic resistance, catheter-associated bacteriuria is generally not treated with antibiotics (22–24). Elimination of *P. mirabilis* by appropriate therapy as soon as it enters the catheterized urinary tract would reduce the incidence of CAUTIs and improve the quality of life of many patients while also reducing the costs of managing the complications of catheter encrustation and blockage (25). Several management and treatment strategies have been used for CAUTIs, including limitation of catheter use, removal of the catheter as soon as possible, maintenance of a closed drainage system, and use of alternative catheter surfaces with anti-infective agents (23, 26). Unfortunately, no single effective strategy for the prevention of CAUTIs has yet been identified.

The purpose of this study was to assess the antimicrobial activities of several essential oils against bacteria involved in CAUTIs. On the basis of these findings, further studies are planned to incorporate these agents into urinary catheter materials to prevent infection. The results of this study show that TTO, terpinen, eugenol, and triclosan possessed antimicrobial activity against the majority of the organisms tested in planktonic growth. Greatly reduced antimicrobial activity was, however, noted when they were used to combat biofilms. The exception to this was eugenol, which retained much of its activity against biofilms cultured in MHB. Recently, eugenol's antibacterial activity against *P. mirabilis* was highlighted by Devi et al., who demonstrated that eugenol altered the cell membrane integrity of *P. mirabilis* (27). Our data also strengthen previous research regarding TTO activity, in particular, terpinen, as the single active constituent of TTO, and its activity *in vivo* (9, 28, 29).

While biofilms were more tolerant to these natural agents than planktonic cells were in our study, sufficient antimicrobial effects were observed to warrant further investigation of the clinical potential of these agents. These observations should encourage clinical studies to examine the effect of washout solutions on the blockage of long-term-catheterized patients. An alternative approach is the incorporation of these agents into biomaterials used in catheter development, thereby generating a catheter surface that could inhibit the growth and swarming of *P. mirabilis*. There is the potential advantage to the use of such agents prophylactically compared to antibiotics (i.e., as they would not encourage the development of antibiotic-resistant organisms).

In conclusion, this study suggests that triclosan, terpinen, and eugenol inhibit the growth and swarming of *P. mirabilis* and may prove clinically useful for the treatment of CAUTIs. However, more work is needed to validate the biocides for washout solutions or their incorporation into urinary catheters.

TABLE 1 Antimicrobial activities of essential oils against planktonic and biofilm growth in MHB and AU

Bacterial strain	Reference or strain type	Inhibitory concn (%, vol/vol) effective against planktonic or biofilm growth											
		Cineole in MHB		TTO in MHB		Terpinen in MHB		Terpinen in AU		Eugenol in MHB		Eugenol in AU	
		Planktonic	Biofilm	Planktonic	Biofilm	Planktonic	Biofilm	Planktonic	Biofilm	Planktonic	Biofilm	Planktonic	Biofilm
Proteus mirabilis B2	30	2	>4	0.5	>4	0.125	>4	4	4	0.25	0.25	0.5	4
P. mirabilis RB6	31	2	>4	0.25	>4	0.125	>4	4	4	0.25	0.5	0.5	4
P. mirabilis NSM6	32	2	>4	0.5	>4	0.125	>4	>4	>4	0.25	0.5	0.5	>4
P. mirabilis NCTC 13376	Reference strain	4	>4	4	>4	0.5	>4	1	4	0.125	1	0.5	4
P. mirabilis HI4320	33	1	>4	0.25	1	0.125	1	4	2	0.06	0.5	0.5	2
P. mirabilis NP6	34	>4	>4	0.5	2	0.125	1	4	4	0.06	0.25	0.5	4
P. mirabilis RB22	Clinical isolate	>4	>4	0.25	1	0.125	1	2	4	0.06	0.5	0.5	4
P. mirabilis urease-ve	Clinical isolate	2	>4	0.25	2	0.125	1	0.25	4	0.06	0.5	0.25	4
Proteus vulgaris H2V	Clinical isolate	1	>4	0.25	2	0.125	1	2	4	0.125	0.5	0.25	4
P. vulgaris NSM19	34	1	>4	0.5	2	0.125	1	4	1	0.25	0.5	0.5	4
P. vulgaris SDM2	31	2	>4	0.5	>4	0.125	>4	4	4	0.25	0.5	0.5	4
Providencia stuartii NSM40	12	1	>4	0.5	>4	0.125	>4	0.06	4	0.25	>4	0.5	4
Escherichia coli NCTC 12923	Reference strain	4	>4	4	>4	0.25	>4	0.5	4	0.125	2	>4	4
Pseudomonas aeruginosa NCTC 10662	Reference strain	>4	>4	4	>4	1	>4	4	2	2	0.5	>4	4
Staphylococcus aureus P10 6/9	31	4	>4	1	>4	0.25	>4	1	4	0.25	4	4	4

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